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ARTICLE

Estimating Abundance and Life History Characteristics of Threatened Wild Snake River Steelhead Stocks by Using Genetic Stock Identification

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Abstract

Assessments of threatened wild Snake River steelhead *Oncorhynchus mykiss* have historically been limited due to a lack of stock-specific information and difficulties in field sampling efforts. We used genetic stock identification (GSI) to estimate the composition of wild adult steelhead migrating past Lower Granite Dam on the Snake River between August 24 and November 25, 2008. Further, we combined genetic data with information on sex, length, age, and run timing to examine for differences in life history or demography among stocks. In total, 1,087 samples collected at the dam were genotyped with 13 standardized steelhead microsatellite loci and a new modified Y-chromosome-specific assay that differentiates sex. A genetic baseline of 66 populations was used to complete GSI of unknown-origin samples from Lower Granite Dam. Large differences in reporting group (stock) contributions were observed for the run as a whole; the Snake River–lower Clearwater River reporting group had the largest single contribution of 36.1% (95% confidence interval [CI] = 30.2–39.7%). Other large contributions were 15.4% (12.8–18.7%) from the upper Clearwater River reporting group and 13.9% (12.5–18.7%) from the lower Salmon River reporting group. Smaller contributions came from the other six reporting groups (Imnaha River: mean = 9.5%, 95% CI = 6.8–13.6%; upper Salmon River: 9.2%, 5.1–11.3%; South Fork Clearwater River: 7.6%, 4.3–8.9%; Middle Fork Salmon River: 5.1%, 3.5–6.4%; South Fork Salmon River: 2.7%, 1.3–3.6%; Elk Creek: 0.5%, 0.0–1.2%). Significant differences in reporting group contributions were observed when samples were grouped according to length, age, and run timing differences. Of the samples analyzed, 372 (34.9%) were identified as males and 694 (65.1%) were identified as females. Our results demonstrate that the GSI methodologies applied to Snake River steelhead have the potential of providing an efficient, minimally intrusive tool for obtaining stock-specific abundance of this threatened distinct population segment. This technology can assist future viability status assessments of Snake River steelhead by contributing to refinements in population delineations, productivity calculations, and annual stock-specific estimation of life history characteristics (e.g., age structure, sex ratio, and run timing).

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Steelhead *Oncorhynchus mykiss* in the Pacific Northwest, USA, have been in decline for the last several decades. In the Columbia River basin, steelhead belong to five distinct population segments (DPSs), all of which are listed as threatened under the Endangered Species Act (U.S. Office of the Federal Register 2011). To assess the extinction risk of salmonid populations and the viability of DPSs, the National Marine Fisheries Service developed the viable salmonid population concept (McElhany et al. 2000). Under this concept, managers attempt to delineate population structure and spatial boundaries, estimate past and present population abundance and growth, and characterize and quantify the diversity of life history characteristics expressed within each DPS. Life history information includes length of freshwater rearing and ocean residency, run timing, age structure at return, size at age, and sex ratio. These assessments contribute to recovery efforts because they allow a better understanding of the mechanisms that have led to population declines and they provide a knowledge base from which to formulate predictions of stocks' responses to different types of management action.

Assessments of the status of Snake River summer-run steelhead have been particularly challenging. The Snake River DPS was originally listed as threatened under the Endangered Species Act in 1997 and encompasses populations that spawn throughout the basin in central Idaho, northeastern Oregon, and southeastern Washington. Formerly, over half of the steelhead produced in the Columbia River basin spawned in Snake River tributaries (Mallet 1974). Raymond (1988) documented that the survival of steelhead emigrating from the Snake River decreased after the construction of dams on the lower Snake River during the late 1960s and early 1970s. There was a period of recovery in the early 1980s, but adult escapement past Lower Granite Dam (Figure 1) into the Snake River basin declined again. While hatchery returns increased, the returns of naturally produced steelhead remained critically low, especially for stocks with a later run timing (Busby et al. 1996). Spawning escapement estimates (and other demographic information) are unavailable for most Snake River steelhead stocks (Busby et al. 1996; Good et al. 2005), and this lack of information presents a persistent challenge to management of the species. Given that steelhead in the Snake River basin spawn on the peak of the spring snowmelt, flow conditions preclude typical monitoring methods, such as weir trapping, spawning observations, and redd counts.

In lieu of more detailed drainage-level, stock-specific information, steelhead that spawn in the Snake River basin have traditionally been assigned to two groups (A-run and B-run) based on the bimodal timing of passage into the Columbia River (as measured at Bonneville Dam) and based on certain life history characteristics (Busby et al. 1996). By definition, A-run steelhead pass Bonneville Dam before August 25 and tend to return after 1 year in the ocean. The B-run steelhead pass Bonneville Dam after August 25, tend to return after 2 years in the ocean, and are thought to be larger at age than A-run steelhead. Migrating adults do not exhibit a bimodal passage distribution at Lower Granite Dam, and A-run and B-run adults are therefore differentiated and enumerated based on length (A-run: $\leq$78 cm; B-run: $>$78 cm; Schrader et al. 2011). In addition to run timing at Bonneville Dam and size differences, the two stocks are believed to also exhibit differences in spawning distribution. The A-run adult steelhead are thought to spawn throughout the Columbia River basin, whereas the B-run steelhead are believed to originate primarily from the Clearwater, Middle Fork Salmon, and South Fork Salmon rivers in Idaho. Putative migration timing and life history characteristics have been used as surrogates for biodiversity in conservation planning for Snake River steelhead. However, the relationship between life history characteristics and passage timing at Bonneville Dam is uncertain (Good et al. 2005). Furthermore, the passage distribution at Bonneville Dam has shifted from bimodal to unimodal in recent years (Robards and Quinn 2002).

Two principal management issues involving Snake River steelhead have arisen in the last several years. First, B-run populations do not appear to be self sustaining (NOAA 2008), and their presence in the drainage has affected Columbia River hydrosystem operation and lower Columbia River fisheries management. In particular, harvest of fall Chinook salmon *O. tshawytscha* is contrained in order to limit impacts to B-run steelhead that are concurrently present in the Columbia River. Secondly, although Snake River B-run steelhead are currently identified as a biologically significant and distinct component of the Snake River evolutionarily significant unit (NOAA 2003), their management is confounded by the lack of a clear and detailed understanding of their actual spawning distribution and evolutionary structure. Nielsen et al. (2009) found that steelhead in Snake River tributaries within Idaho exhibited a complicated pattern of genetic structure, with populations grouping genetically according to drainage locality rather than simply to A-run and B-run designations.

These types of management issues can potentially be addressed through genetic stock identification (GSI). In GSI analysis, reference populations from all suspected contributing stocks are screened with multilocus genetic markers. By use of statistical algorithms, these populations are then grouped or "clustered" together into reporting groups based on genetic similarities. When mixtures of fish of unknown origin are genotyped at the same sets of genetic markers, it is possible to estimate the proportion of each reporting group represented in the mixture (Shaklee et al. 1999; Anderson et al. 2008). A variety of Pacific salmonids, including Chinook salmon, sockeye salmon *O. nerka*, chum salmon *O. keta*, and steelhead, have been researched and managed by using GSI technologies (Beacham et al. 1999, 2000, 2008a, 2008b; Habicht et al. 2007). Previous genetic studies have indicated that steelhead in the Snake River basin exhibit significant genetic structuring at the drainage level (Moran 2003; Nielsen et al. 2009), and GSI procedures have already been used successfully to identify the origin of postspawn steelhead at Lower Granite Dam (Narum et al. 2008). In the present study, we used similar GSI methods to identify the stock

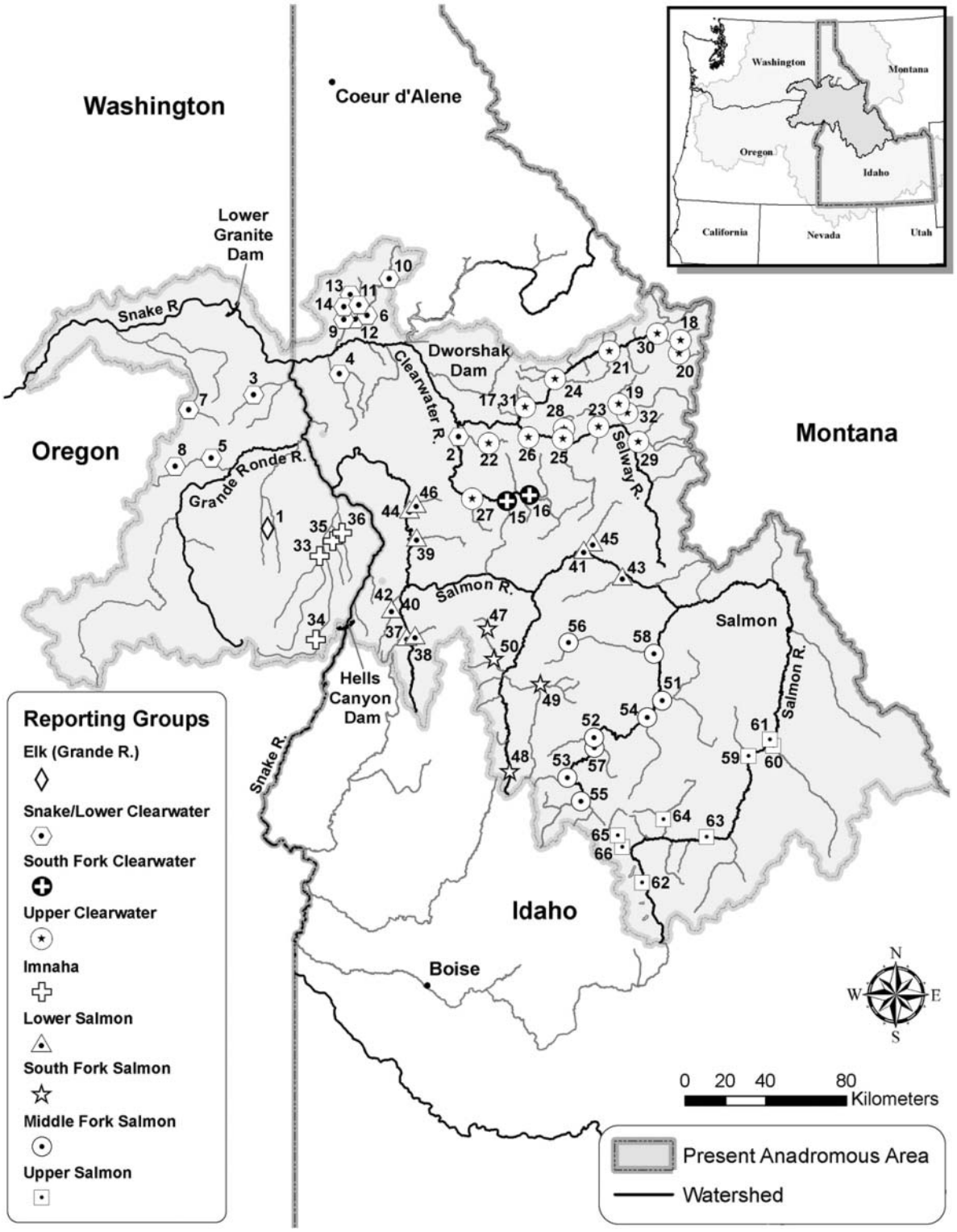

FIGURE 1. The 66 Snake River steelhead populations that served as baseline populations for genetic stock identification (GSI) mixture analyses. Numbers correspond to the site numbers defined in Table 1. Symbols refer to the nine genetic clusters that were identified in Bayesian Analysis of Population Structure software and that were used for GSI reporting groups.

composition of adult wild steelhead migrating past Lower Granite Dam and we combined genetic data with sex, length, age, and run timing information to evaluate demographic similarities and differences among stocks.

METHODS

Snake River genetic baseline.—A genetic baseline of 66 wild, anadromous Snake River basin steelhead collections was available as part of a multilaboratory, collaborative effort to build a standardized coastwide microsatellite baseline for steelhead (Blankenship et al. 2011). All of the collections (3,803 individuals; Table 1; Figure 1) were previously genotyped with a standardized set of 13 microsatellite loci (Stephenson et al. 2009).

To examine genetic relationships among the baseline collections, genetic chord distances (Cavalli-Sforza and Edwards 1967) between all collections were estimated by using GENDIST in PHYLIP version 3.5 (Felsenstein 1993). To help visualize genetic relationships, a neighbor-joining dendrogram was generated from chord distances with the program FITCH in PHYLIP using a bootstrapping algorithm. Bootstrap replicates of 1,000 iterations were attained with SEQBOOT, and a consensus tree was formed with CONSENSE in PHYLIP. The dendrogram was edited and visualized by using TreeGraph 2 (Stöver and Müller 2010).

To assess the appropriate number and population composition of reporting groups for GSI analyses, baseline samples were analyzed with Bayesian Analysis of Population Structure (BAPS) version 5.3 (Corander et al. 2008). The BAPS software assigns samples to K clusters by using a partition-based mixture model that minimizes deviations from Hardy–Weinberg equilibrium and linkage equilibrium within each cluster. Simulated data sets have shown that BAPS can infer the correct number of subpopulation clusters even at low levels of differentiation (Latch et al. 2006). We used the "clustering of groups of individuals" option in BAPS with a predefined maximum K of 66 (corresponding to the total number of collections). We repeated the run 10 times to check the stability of the results. The best clustering solution ("correct" number of reporting groups) was chosen based on the largest log marginal likelihood value from all runs. To describe genetic differentiation among clusters, we calculated pairwise Nei's standard distance (Nei 1972) in BAPS.

To evaluate the potential accuracy of selected reporting groups for GSI, we followed the recommended methods of Anderson et al. (2008) in using the program ONCOR (Kalinowski et al. 2007) to perform 100% simulations. These procedures test each population under the scenario that the mixture solely consists of individuals from that population. A population is generally considered to be highly identifiable if allocation to the correct reporting group is 90% or greater (Seeb et al. 2007). The number of mixtures to generate for each population was set at 1,000, with a mixture sample size of 400. Simulated baseline sample sizes were the same as in the actual baseline.

Trapping, sampling, and age assignment.—Wild adult steelhead were captured at the Lower Granite Dam adult trapping facility (Harmon 2003; Figure 1) from August 24 to November 25, 2008 (Figure 2), coinciding with the collection of fall

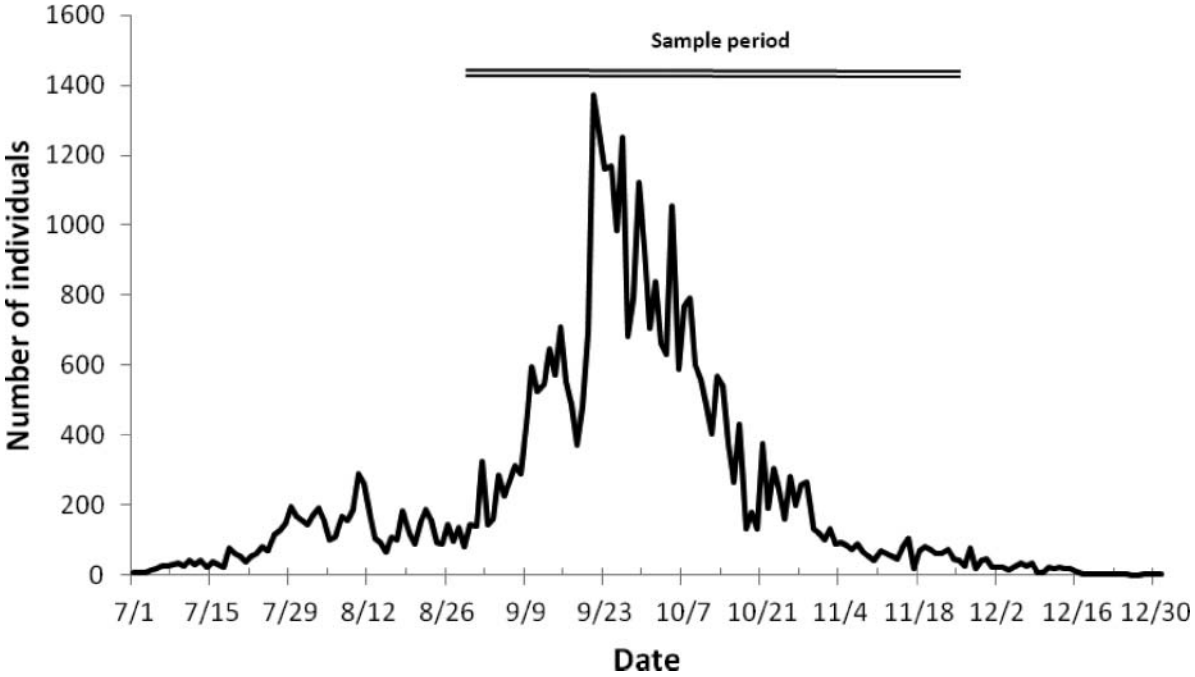

FIGURE 2. Number of wild adult steelhead that passed Lower Granite Dam in 2008. The period when samples were collected (August 24–November 25, 2008) is denoted by the horizontal bar.

TABLE 1. Steelhead populations and corresponding site numbers in the Snake River basin, presented with sample size per population (N), observed heterozygosity (H_O), expected heterozygosity (H_E), and average number of alleles observed per locus (A). For each population, the correct assignment back to reporting group (reporting group accuracy [RGA]) from 100% simulations in ONCOR is presented with lower and upper 95% confidence limits (CLs; RGA = the proportion of simulated fish, in a 100% mixture of fish from a given population, that were correctly assigned back to that population). Genotyping agencies were the Columbia River Inter-Tribal Fish Commission (CRITFC), the Idaho Department of Fish and Game (IDFG), and the Northwest Fisheries Science Center (NWFSC, National Oceanic and Atmospheric Administration Fisheries).

Collection	Site number	Agency	N	H_O	H_E	A	RGA	Lower 95% CL	Upper 95% CL
Elk Creek (Grande Ronde River) reporting group									
Elk Creek	1	NWFSC	96	0.77	0.76	9.9	0.96	0.93	0.98
Snake River–Lower Clearwater River reporting group									
Cottonwood Creek	2	NWFSC	96	0.77	0.78	11.3	0.94	0.91	0.97
Asotin Creek	3	NWFSC	110	0.79	0.80	13.1	0.87	0.82	0.92
Mission Creek	4	CRITFC	51	0.76	0.76	10.5	0.96	0.94	0.99
Crooked Creek	5	NWFSC	141	0.78	0.78	12.6	0.96	0.93	0.99
Lower East Fork Potlatch River	6	NWFSC	41	0.78	0.77	9.5	0.97	0.95	0.99
Tucannon River	7	NWFSC	74	0.78	0.79	11.9	0.88	0.83	0.93
Wenaha River	8	NWFSC	94	0.77	0.78	11.8	0.91	0.87	0.95
Little Bear Creek	9	IDFG	42	0.75	0.77	10.3	0.90	0.85	0.94
Upper East Fork Potlatch River	10	IDFG	62	0.74	0.75	10.1	0.97	0.95	0.99
Big Bear Creek	11	IDFG	20	0.75	0.76	8.9	0.89	0.84	0.93
Little Bear Creek	12	IDFG	11	0.71	0.76	6.7	0.93	0.89	0.96
Big Bear Creek	13	IDFG	12	0.69	0.73	6.6	0.78	0.71	0.83
Little Bear Creek	14	CRITFC	50	0.80	0.76	9.2	0.98	0.97	1.00
South Fork Clearwater River reporting group									
Tenmile Creek	15	IDFG	47	0.77	0.74	8.5	0.96	0.93	0.98
Crooked River	16	IDFG	80	0.73	0.73	9.6	0.93	0.89	0.96
Upper Clearwater River reporting group									
Canyon Creek	17	CRITFC	34	0.77	0.74	8.2	0.99	0.98	1.00
Storm Creek	18	CRITFC	39	0.75	0.73	8.1	1.00	0.99	1.00
North Fork Moose Creek	19	CRITFC	50	0.73	0.73	9.2	0.99	0.98	1.00
Colt Creek	20	CRITFC	58	0.72	0.71	8.3	1.00	0.99	1.00
Lake Creek	21	CRITFC	52	0.74	0.72	8.8	1.00	0.99	1.00
Clear Creek	22	CRITFC	45	0.74	0.75	9.5	0.91	0.87	0.94
Three Links Creek	23	CRITFC	57	0.78	0.74	8.8	1.00	0.99	1.00
Fish Creek	24	NWFSC	80	0.75	0.75	10.2	0.99	0.98	1.00
Gedney Creek	25	NWFSC	114	0.76	0.75	10.7	0.98	0.96	1.00
O'Hara Creek	26	IDFG	47	0.75	0.76	9.7	0.97	0.94	0.99
Johns Creek	27	IDFG	31	0.74	0.75	9.5	0.75	0.69	0.80
Gedney Creek	28	IDFG	46	0.73	0.75	9.3	0.98	0.96	1.00
Bear Creek	29	IDFG	45	0.78	0.76	8.5	0.99	0.98	1.00
Crooked Fork Lochsa River	30	IDFG	47	0.75	0.75	8.7	1.00	0.98	1.00
Canyon Creek	31	IDFG	47	0.73	0.73	9.6	0.94	0.91	0.97
North Fork Moose Creek	32	IDFG	47	0.74	0.76	8.6	0.99	0.99	1.00
Imnaha River reporting group									
Camp Creek	33	NWFSC	136	0.80	0.77	11.0	0.98	0.96	1.00
Gumboot Creek	34	NWFSC	93	0.78	0.77	9.8	0.97	0.94	0.99
Horse Creek	35	NWFSC	117	0.77	0.78	11.6	0.91	0.87	0.95
Lightning Creek	36	NWFSC	67	0.76	0.78	9.9	0.82	0.77	0.88
Lower Salmon River reporting group									
Boulder Creek	37	IDFG	47	0.77	0.76	10.1	0.93	0.89	0.97
Hazard Creek	38	IDFG	44	0.76	0.78	11.2	0.69	0.63	0.76

TABLE 1. (Continued).

Collection	Site number	Agency	N	H_O	H_E	A	RGA	Lower 95% CL	Upper 95% CL
Slate Creek	39	IDFG	47	0.77	0.79	10.9	0.88	0.83	0.92
Rapid River	40	IDFG	266	0.75	0.76	12.5	0.98	0.96	0.99
Bargamin Creek	41	IDFG	45	0.77	0.78	9.3	0.93	0.90	0.97
Rapid River	42	NWFSC	43	0.76	0.75	9.1	0.99	0.97	1.00
Chamberlain Creek	43	CRITFC	64	0.78	0.76	10.6	0.88	0.82	0.92
Whitebird Creek	44	CRITFC	58	0.76	0.78	9.6	0.96	0.93	0.98
Bargamin Creek	45	NWFSC	45	0.78	0.77	9.3	0.87	0.82	0.92
Whitebird Creek	46	NWFSC	50	0.76	0.77	10.2	0.80	0.74	0.85
South Fork Salmon River reporting group									
Upper Secesh River	47	NWFSC	28	0.70	0.71	6.7	0.99	0.98	1.00
Stolle Meadows	48	NWFSC	44	0.72	0.72	8.2	0.94	0.91	0.97
East Fork South Fork Salmon River	49	IDFG	46	0.77	0.75	8.3	0.91	0.87	0.94
Lower Secesh River	50	IDFG	45	0.70	0.73	8.3	0.95	0.92	0.98
Middle Fork Salmon River reporting group									
Camas Creek	51	CRITFC	52	0.75	0.75	9.5	0.92	0.88	0.95
Pistol Creek	52	CRITFC	23	0.76	0.73	7.6	0.90	0.85	0.94
Sulphur Creek	53	CRITFC	53	0.77	0.72	8.1	0.98	0.96	0.99
Loon Creek	54	CRITFC	59	0.73	0.73	8.8	0.95	0.92	0.98
Marsh Creek	55	CRITFC	57	0.74	0.73	7.7	0.99	0.98	1.00
Upper Big Creek	56	NWFSC	42	0.77	0.77	9.2	0.83	0.78	0.88
Rapid River	57	IDFG	45	0.70	0.72	8.5	0.91	0.87	0.94
Lower Big Creek	58	IDFG	47	0.75	0.74	7.1	1.00	0.99	1.00
Upper Salmon River reporting group									
Morgan Creek	59	IDFG	45	0.80	0.81	11.4	0.86	0.81	0.90
Pahsimeroi River	60	IDFG	41	0.81	0.81	10.8	0.77	0.72	0.83
Pahsimeroi River	61	IDFG	47	0.79	0.80	10.5	0.89	0.84	0.93
Sawtooth Weir	62	IDFG	29	0.77	0.78	9.6	0.76	0.70	0.82
Squaw Creek	63	IDFG	21	0.79	0.79	9.2	0.67	0.60	0.73
West Fork Yankee Fork Salmon River	64	IDFG	47	0.83	0.80	10.2	0.91	0.88	0.95
Upper Valley Creek	65	NWFSC	25	0.78	0.77	7.5	0.92	0.88	0.96
Lower Valley Creek	66	NWFSC	19	0.83	0.79	8.3	0.78	0.72	0.84

Chinook salmon broodstock for the Lyons Ferry Fish Hatchery. The trapping rate for steelhead was dependent upon the trapping rate for fall Chinook salmon, which varied between 10% and 20%. Although most of the hatchery-origin steelhead have a clipped adipose fin, thus allowing for differentiation from wild fish, some are misclipped or are intentionally released unclipped for supplementation purposes. At the adult trapping facility, unclipped hatchery steelhead are identified by the presence of dorsal or ventral fin erosion (Schrader et al. 2011). In 2008, 13.0% of hatchery steelhead passing Lower Granite Dam were unclipped (Schrader et al. 2011). Sampled wild adults were measured for fork length to the nearest centimeter, and scales were collected to determine age. Tissue samples were taken from the anal fin by using a tissue punch and were stored in 100% nondenatured ethanol. Fish were subsampled from the total number of wild-origin samples collected at the adult trap to maintain an overall sample rate of approximately 5%.

Freshwater and saltwater ages were assigned to each fish based on scale pattern analysis (Davis and Light 1985). Two technicians independently viewed each image to assign ages. Freshwater ages were assigned using a $4\times$ magnified image, and saltwater ages were assigned using a $1.25\times$ magnified image. The criterion for a saltwater annulus was the crowding of circuli outside of the check for ocean entry. Freshwater annuli were defined by the "pinching" or "cutting over" of circuli within the freshwater zone in the center of the scale. If there was no age consensus between the two readers, a third reader viewed the image; all readers then collectively examined the image to resolve their differences before a final age was assigned. If a

consensus among the three readers was not attained, the scale sample was excluded from further analysis.

Genotyping and genetic stock identification.—A Nexttec Genomic DNA Isolation Kit was used to extract DNA from tissue samples in accordance with the manufacturer's instructions. Samples were amplified with the 13 standardized microsatellite loci (Stephenson et al. 2009). Specific PCR amplification protocols for all loci, as well as thermal cycling conditions, are available from the corresponding author upon request. Descriptive statistics, including the number of alleles per locus, observed heterozygosity, and expected heterozygosity, were estimated for each baseline collection by using the Microsatellite Toolkit for Microsoft Excel (Park 2001).

In addition to the 13 microsatellite loci, all samples were also screened with Y-chromosome-specific assays that differentiate sex in steelhead. Details of assay configuration and the screening performed on known-sex samples to verify accuracy are described in the Appendix.

To address questions of abundance and demography for the identified stocks, we integrated the genetic data with sex, length, age, and migration timing data from adults sampled at Lower Granite Dam. Putative A-run and B-run steelhead are distinguished on the basis of length ($\leq$78 or $>$78 cm), age (1 saltwater versus older), and migratory timing (early versus late). Mixture analyses were performed with ONCOR software in different arrangements to estimate stock components under five different scenarios: (1) for the entire wild run of steelhead (all samples grouped together), (2) by sex (males and females separated), (3) by size (mixtures grouped by length: $\leq$78 or $>$78 cm), (4) by run timing (mixtures grouped as early [August 24–September 22]; middle [September 23–October 23]; and late [October 24–November 25]), and (5) by total age (3, 4, and 5 years). Separate mixtures were also run with 4-year-old fish separated into two age-classes as defined by years in freshwater and years in saltwater (freshwater: saltwater = 2:2 or 3:1). A 95% confidence interval (CI) for stock composition estimates to each reporting group was estimated by bootstrapping the baseline and mixtures for 1,000 iterations as implemented in ONCOR (Kalinowski et al. 2007).

RESULTS

Snake River Genetic Baseline

Basic descriptive statistics for baseline populations are shown in Table 1. More comprehensive summaries of tests for Hardy–Weinberg equilibrium, linkage disequilibrium, population diversity, and population differentiation were published as part of a larger collaborative effort to describe the influence of landscape on the genetic structure of steelhead throughout the Columbia River basin (Blankenship et al. 2011). The neighbor-joining dendrogram based on Cavalli-Sforza and Edwards' (1967) genetic chord distances generally supported genetic population structuring at the subbasin or drainage scale (Figure 3). Bootstrap support greater than 50% was observed for population groupings in the Clearwater, Middle Fork Salmon, South Fork Salmon, upper Salmon, Imnaha, and Grande Ronde rivers. Genetic relationships among populations in tributaries to the main-stem Snake, Little Salmon, and main-stem Salmon rivers were less clear, especially between populations that were found lower in these drainages.

Results of group-level mixture analysis on baseline populations with BAPS indicated that the K in the optimal partition was 9, with a log marginal likelihood of 191,524.04 and a posterior probability of 1. The nine clusters were used as reporting groups for subsequent mixed-stock analyses: (1) Elk Creek (Grande Ronde River), (2) Snake River and lower Clearwater River, (3) South Fork Clearwater River, (4) upper Clearwater River, (5) Imnaha River, (6) lower Salmon River, (7) South Fork Salmon River, (8) Middle Fork Salmon River, and (9) upper Salmon River. Clusters generally followed the genetic structuring observed in the neighbor-joining dendrogram and consisted of geographically proximate populations (Figure 1). One exception was Johns Creek (South Fork Clearwater River subbasin), which clustered apart from neighboring populations and instead grouped with populations from the upper Clearwater River (Lochsa River and Selway River drainages). The Snake River–lower Clearwater River cluster encompassed samples from multiple drainages, including the Tucannon River, lower main-stem Clearwater River (below the North Fork Clearwater River), Asotin Creek, and lower Grande Ronde River. The geographic center of this large, multidrainage cluster lies approximately at the confluence of the Snake and Clearwater rivers. Another large, multidrainage cluster was associated with the confluence of the Salmon and Little Salmon rivers and contained samples from the main-stem Salmon River watershed above the Little Salmon River confluence (Bargamin and Chamberlain creeks), from the Little Salmon River (Rapid River, Boulder Creek, and Hazard Creek), and from the main-stem Salmon River below the Little Salmon River confluence (Slate and Whitebird creeks). All but one of the genetic clusters contained multiple populations. The exception was Elk Creek in the Joseph Creek drainage (Grande Ronde River). Pairwise estimates of Nei's genetic distance between clusters ranged from a low of 0.030 (Imnaha River versus Snake River–lower Clearwater River) to a high of 0.192 (South Fork Clearwater River versus Elk Creek; Table 2). The two clusters with the highest average pairwise genetic distances were the upper Clearwater River (0.122) and the South Fork Clearwater River (0.134), and the two clusters with the lowest average pairwise genetic distances were the lower Salmon River (0.072) and the Snake River–lower Clearwater River (0.062).

Results from 100% simulations in ONCOR using the nine reporting groups indicated that seven groups exhibited over 90% mean correct allocation back to reporting group across all populations (Table 3). The two reporting groups that exhibited less than 90% correct allocation were the upper Salmon River (81.8%) and lower Salmon River (88.3%) groups. For the upper Salmon River reporting group, the largest mean misallocation

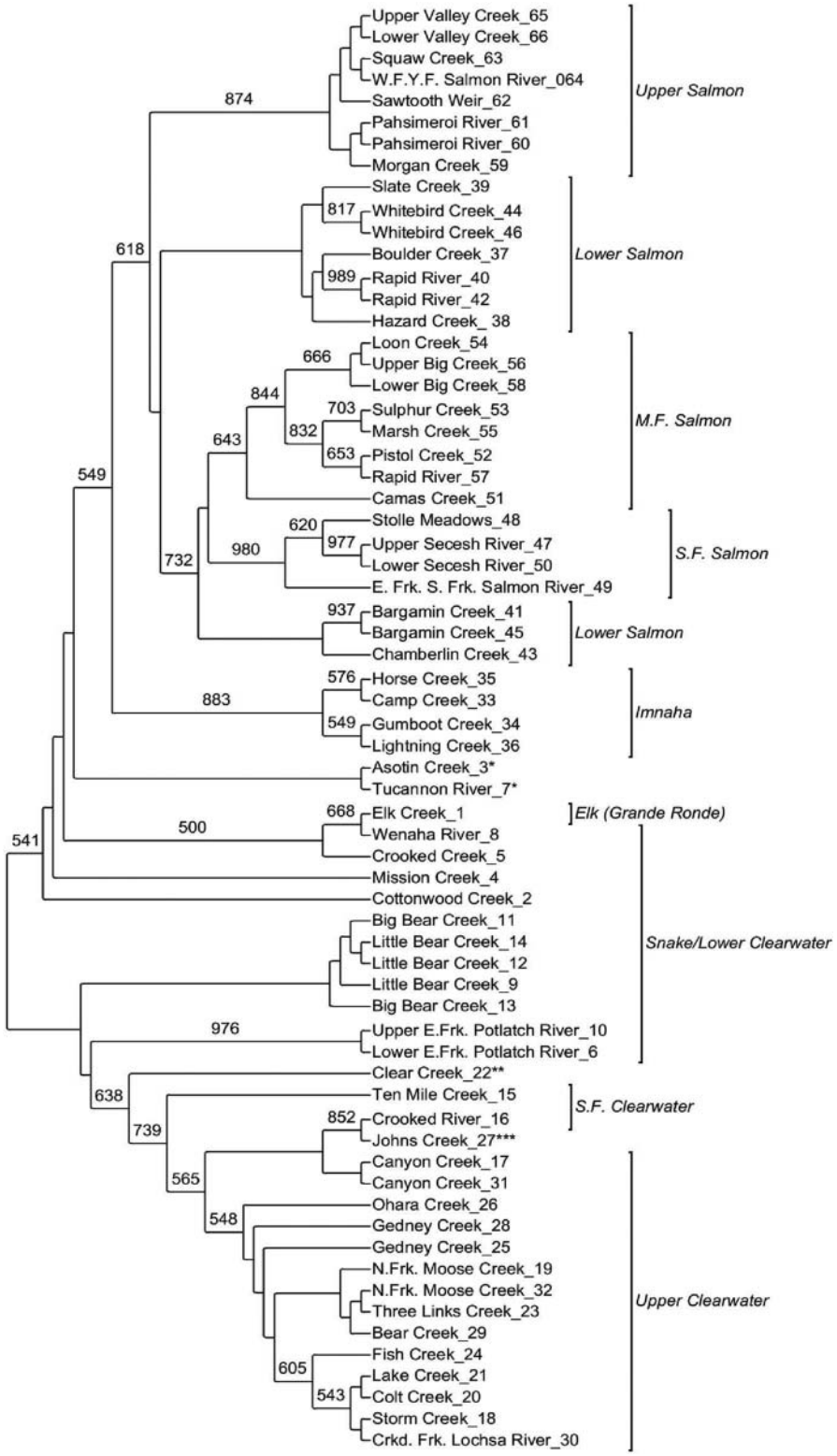

FIGURE 3. Unrooted neighbor-joining dendrogram (Fitch–Margoliash tree based on genetic chord distances [Cavalli-Sforza and Edwards 1967]), showing genetic relationships among the 66 Snake River steelhead baseline sample collections (site number, also defined in Table 1, is presented after each site name). Bootstrap values are only listed if they exceeded 50% of the total iterations (1,000). Italicized names next to brackets refer to cluster (reporting group) designations identified by Bayesian Analysis of Population Structure software (M.F. = Middle Fork; S.F. = South Fork; W.F.Y.F. = West Fork Yankee Fork; Crkd. Frk. = Crooked Fork; * = Asotin Creek_3 and Tucannon River_7 are part of the Snake River–lower Clearwater River reporting group; ** = Clear Creek_22 is part of the upper Clearwater River reporting group; *** = Johns Creek_27 is part of the Snake River–lower Clearwater River reporting group).

TABLE 2. Pairwise Nei's genetic distance estimates for the nine genetic clusters (reporting groups) of steelhead identified in Bayesian Analysis of Population Structure software. Clusters are (1) Elk Creek (Grande Ronde River), (2) Snake River–lower Clearwater River, (3) South Fork Clearwater River, (4) upper Clearwater River, (5) Imnaha River, (6) lower Salmon River, (7) South Fork Salmon River, (8) Middle Fork Salmon River, and (9) upper Salmon River.

	Cluster number							
Cluster number	1	2	3	4	5	6	7	8
2	0.061							
3	0.192	0.094						
4	0.180	0.079	0.062					
5	0.077	0.030	0.137	0.122				
6	0.086	0.044	0.136	0.125	0.039			
7	0.135	0.087	0.185	0.166	0.083	0.064		
8	0.121	0.063	0.149	0.132	0.067	0.035	0.078	
9	0.102	0.038	0.116	0.111	0.048	0.045	0.105	0.086

was to the lower Salmon River reporting group (7.7%). For the lower Salmon River reporting group, the largest mean misallocation was to the Snake River–lower Clearwater River reporting group (4.4%).

Trapping, Sampling, and Age Assignment

Annually, most of the migrating steelhead pass Lower Granite Dam during September and October (Figure 2); during spawn year (SY) 2009, approximately 86% (22,157) of the total

TABLE 3. Results of 100% simulations in ONCOR. The percent allocation of steelhead back to reporting group averaged across all populations is shown. Mean percent correct allocations (in bold italics) are shown along the diagonal.

	Reporting group allocation								
Actual reporting group	Elk Creek (Grande Ronde River)	Snake River–lower Clearwater River	South Fork Clearwater River	Upper Clearwater River	Imnaha River	Lower Salmon River	South Fork Salmon River	Middle Fork Salmon River	Salmon River
Elk Creek (Grande Ronde River)	***95.3***	3.1	0.0	0.1	0.6	0.5	0.0	0.1	0.2
Snake River–lower Clearwater River	0.4	***90.8***	0.2	2.6	1.9	2.2	0.1	0.3	1.5
South Fork Clearwater River	0.0	0.7	***94.0***	4.8	0.1	0.2	0.0	0.1	0.1
Upper Clearwater River	0.0	1.2	1.6	***96.3***	0.2	0.3	0.1	0.2	0.1
Imnaha River	0.1	5.2	0.0	0.3	***91.4***	1.7	0.2	0.3	0.8
Lower Salmon River	0.1	4.4	0.0	0.5	1.5	***88.3***	0.6	1.7	2.9
South Fork Salmon River	0.0	0.5	0.0	0.3	0.6	3.4	***94.2***	0.9	0.1
Middle Fork Salmon River	0.0	0.7	0.0	0.4	0.3	5.0	0.4	***93.1***	0.2
Upper Salmon River	0.1	7.3	0.1	0.5	2.3	7.7	0.1	0.2	***81.8***

TABLE 4. Number of steelhead individuals that were assigned freshwater and ocean ages among fish sampled at Lower Granite Dam during 2008 (X = only the ocean age was assigned).

	Freshwater age					
Ocean age	X	1	2	3	4	5
1	13	5	175	204	27	1
2	35	2	279	158	25	0
3	5	0	20	19	0	0

escapement passed the dam during our sampling period. The estimate of total escapement of wild steelhead that migrated past Lower Granite Dam for the entire run year (between July 1, 2008, and June 30, 2009) was 25,764 (95% CI = 20,301–31,673; Schrader et al. 2011).

In total, 998 scale samples were viewed in an attempt to assign ages (Table 4). Of these, 968 were assigned an ocean age, 915 were assigned both freshwater and ocean ages, and 30 could not be aged. Of 29 fish with known ocean ages from passive integrated transponder tags, 28 fish were aged accurately; thus, the accuracy of age assignments was estimated at 97%. Freshwater ages ranged from 1 to 5 years, and ocean ages ranged from 1 to 3 years (Table 4). More than half (543/968 = 56.1%) of the fish had spent a minimum of 2 years in the ocean, which was previously believed to occur predominantly in B-run stocks. Nearly all of the fish had smolted at 2 or 3 years of age (908/915 = 99.2%). This is in sharp contrast to Snake River hatchery steelhead, which almost exclusively undergo smoltification after 1 year in freshwater (PTAGIS 2011). Total ages at the time of sampling ranged from 2 to 6 years. The length distribution was bimodal, and the proportion of older and larger fish increased over the course of the run (Figure 4).

A total of 1,092 samples were extracted and genotyped. Of these, 1,076 (98.5%) samples yielded complete genotypes ($\geq$10 loci), and only those samples were used in GSI analyses. Because biological information (sex, length, and age) for some samples was incomplete, some mixture analyses were run with a total sample size less than 1,076 (all $\geq$ 914).

The largest contributor to the aggregate run passing Lower Granite Dam was the Snake River–lower Clearwater River reporting group, with a mean of 36.1% (95% CI = 30.2–39.7%; Figure 5a), followed by the upper Clearwater River reporting group (mean = 15.4%; 95% CI = 12.8–18.7%) and the lower Salmon River reporting group (13.9%; 95% CI = 12.5–18.7%). The remaining reporting groups each contributed less than 10% to the overall mixture. Mean contributions were 9.5% (95% CI = 6.8–13.6%) from the Imnaha River, 9.2% (5.1–11.3%) from the upper Salmon River, 7.6% (4.3–8.9%) from the South Fork Clearwater River, 5.1% (3.5–6.4%) from the Middle Fork Salmon River, 2.7% (1.3–3.6%) from the South Fork Salmon River, and 0.5% (0.0–1.2%) from Elk Creek.

Sex ratio was female biased for the 1,066 samples in which sex was identified using the genetic sex assay. Of the 1,066 samples, 372 were males (34.9%) and 694 were females (65.1%). Mixture analyses with samples grouped by sex did not identify any significant differences in reporting group contributions between males and females (all comparisons yielded overlapping 95% CIs; Figure 5b).

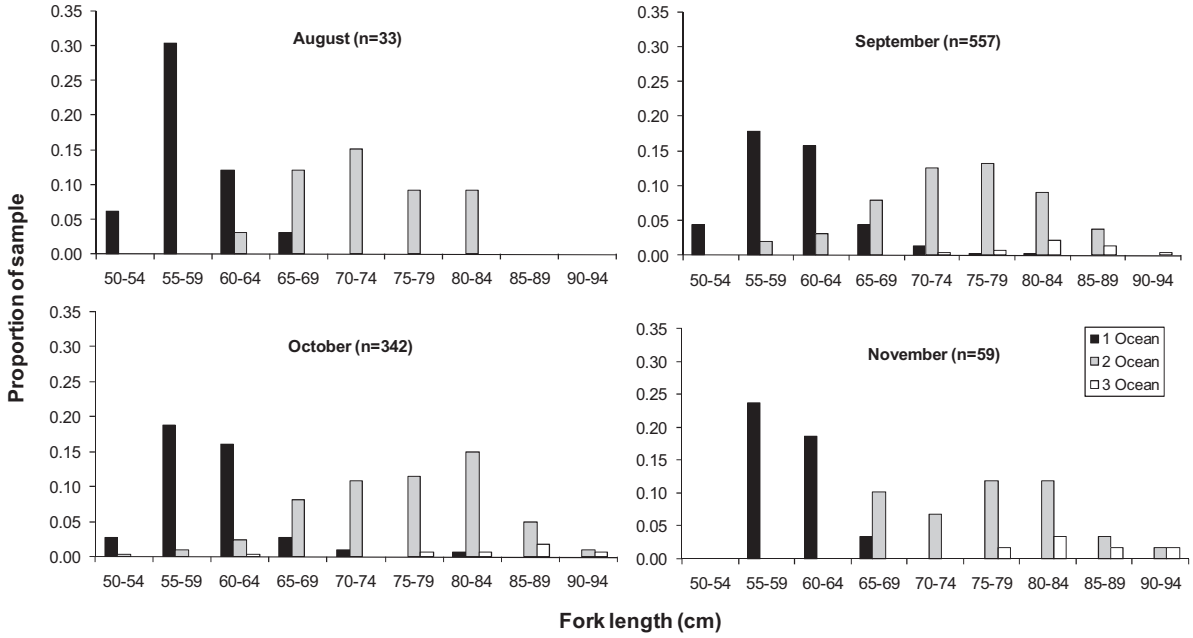

FIGURE 4. Length frequency of steelhead by ocean age for each month of collection at Lower Granite Dam during 2008 (ages were determined from scale samples).

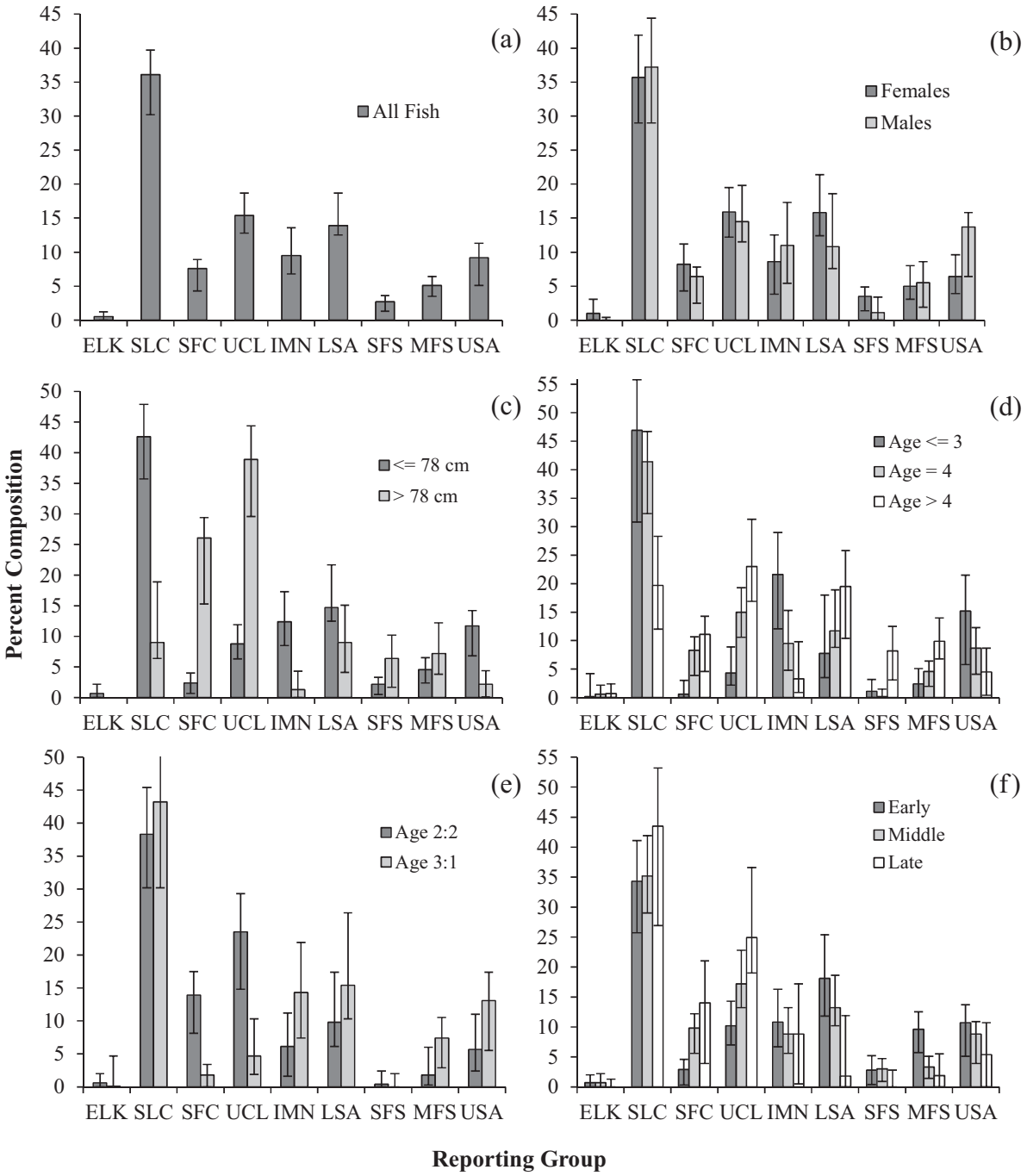

FIGURE 5. Estimated percent contributions (±95% confidence interval) from nine reporting groups for mixtures of adult steelhead passing Lower Granite Dam: **(a)** all adults, **(b)** adults grouped by sex, **(c)** adults grouped by fork length (≤78 or >78 cm), **(d)** adults grouped by combined age (years; freshwater age + ocean age), **(e)** 4-year-old adults grouped by freshwater years: ocean years (age 2:2 or age 3:1), and **(f)** adults grouped by run timing. Reporting groups are Elk Creek (Grande Ronde River; ELK), Snake River–lower Clearwater River (SLC), South Fork Clearwater River (SFC), upper Clearwater River (UCL), Imnaha River (IMN), lower Salmon River (LSA), South Fork Salmon River (SFS), Middle Fork Salmon River (MFS), and upper Salmon River (USA).

Stock composition of each reporting group when analyzed by fork length was noticeably varied across mixtures (Figure 5c). For 78-cm and smaller adults ($N = 767$), the largest contributor to the mixture was the Snake River–lower Clearwater River reporting group at 42.6% (95% CI = 35.7–47.9%), followed by the lower Salmon River (14.7%; 12.5–21.7%), Imnaha River (12.4%; 8.4–17.3%), and upper Salmon River (11.7%; 6.8–14.2%) reporting groups. The remaining reporting groups each

contributed less than 10% to the overall mixture of smaller fish. For adults larger than 78 cm ($N = 229$), the greatest contributor was the upper Clearwater River reporting group (mean = 38.9%; 95% CI = 29.4–44.4%), followed by the South Fork Clearwater River reporting group (26.1%; 15.3–29.4%). All of the remaining reporting groups each contributed less than 10% to the overall mixture of larger adults. Besides the upper Clearwater River and South Fork Clearwater River groups, the South Fork Salmon River and Middle Fork Salmon River were the only other reporting groups for which overall contributions were greater in the B-run mixture (>78 cm) than in the A-run mixture (≤78 cm).

We observed different patterns of stock composition when samples were grouped by combined age (freshwater age + ocean age; Figure 5d). The largest contributors to the mixture of age-3 and younger adults ($N = 182$) were the Snake River–lower Clearwater River (mean = 46.9%; 95% CI = 30.8–55.9%), Imnaha River (21.58%; 12.1–29.0%), and upper Salmon River (15.2%; 5.8–21.5%) reporting groups. However, the contribution of these three reporting groups to the mixture of age-4 adults ($N = 483$) was substantially lower, and their contribution to the mixture of age-5 and older adults ($N = 250$) exhibited a further decrease. Moreover, both the Imnaha River (mean = 3.3%; 95% CI = 0.9–9.8%) and the upper Salmon River (4.5%; 4.1–8.7%) reporting groups were among the lowest contributors to age-5 and older adults. The opposite trend was observed among the remaining reporting groups, for which the contributions increased as mixture ages increased. The largest contributor to the mixture of age-5 and older adults was the upper Clearwater River, followed by the Snake River–lower Clearwater River (mean = 19.7%; 95% CI = 12.0%–28.3), lower Salmon River (19.5%; 10.4–25.8%), and South Fork Clearwater River (11.1%; 4.6–14.3%). The remaining reporting groups contributed less than 10% to the overall mixture of age-5 and older adults, although all made their highest contributions to this age-group.

Differences in reporting group contributions were also observed when 4-year-old adults were separated into their two respective age-classes (freshwater years: saltwater years = 2:2 or 3:1; Figure 5e). The South Fork Clearwater River and upper Clearwater River reporting groups contributed significantly more to the mixture of age-2:2 adults (South Fork Clearwater: mean = 13.9%, 95% CI = 7.2–17.0%; upper Clearwater: mean = 23.5%, 95% CI = 15.6–29.7%) than to the mixture of age-3:1 adults (South Fork Clearwater: mean = 1.8%, 95% CI = 0.0–3.3%; upper Clearwater: mean = 4.7%, 95% CI = 2.3–11.0%). Contrasting results were observed among the Imnaha, lower Salmon, Middle Fork Salmon, and upper Salmon River reporting groups, which contributed more to the age-3:1 mixture than to the age-2:2 mixture.

Drainage-specific trends were apparent when separating samples by run timing (Figure 5f). Reporting groups associated with the Clearwater River drainage (Snake River–lower Clearwater River, South Fork Clearwater River, and upper Clearwater River) exhibited a trend of increasing contributions to their respective mixtures as the run progressed. Conversely, reporting groups associated with the Salmon River (lower Salmon River, South Fork Salmon River, Middle Fork Salmon River, and upper Salmon River) all exhibited a trend of decreasing contributions throughout the run. No clear patterns of increasing or decreasing contributions during the run were observed for the Elk Creek or Imnaha River reporting group.

DISCUSSION

Our results are consistent with more recent genetic investigations indicating that steelhead within and outside the Snake River basin exhibit a complicated pattern of genetic structuring that is partitioned at multiple spatial scales according to environmental and habitat parameters and the influence of hatchery introgression (Nielsen et al. 2009; Blankenship et al. 2011). Our reporting groups were generally correlated with single, terminal river drainages situated at higher elevations and in areas that have been managed for wild populations. However, we also observed large reporting clusters that encompassed main-stem areas and multiple drainages, suggesting interdrainage gene flow. This may due to similarities in elevation and geology within these areas, leading to similarities in life history timing (emigration and spawning) that would permit successful straying among drainages, thereby reducing population structure. Introgression from hatchery steelhead may have also influenced genetic structure in the main-stem Salmon River, Little Salmon River, and lower Snake River areas (Nielsen et al. 2009), which correspond to the Snake River–lower Clearwater River and lower Salmon River clusters we identified in this study.

The reporting groups we identified were delineated strictly by genetic relationships and in many instances do not follow the populations identified by the Interior Columbia Basin Technical Recovery Team (ICBTRT; ICBTRT 2003). The ICBTRT designations were based largely on a drainage-level geographic hierarchy supplemented with genetic information (Moran and Waples 2004). However, the available genetic data at that time had limited representation from the Idaho portion of the basin, and there was a paucity of data on spawning distributions, natural levels of straying, and hatchery influence within the basin. Results from this study indicate that the construction of fine-scale genetic baselines will contribute to efforts to refine population delineations in the Snake River evolutionarily significant unit for viability assessments.

Using the nine identified reporting groups, we were for the first time able to apportion the adult steelhead escapement to the Snake River basin according to geographic stock structure. Such abundance data were not available to support earlier conservation assessments (Busby et al. 1996; Good et al. 2005). During the SY 2009 escapement period, the largest proportion of adults passing Lower Granite Dam were from the Snake River–lower Clearwater River reporting group, and the remaining contributions ranged from 2.7% (95% CI = 1.3–3.6%; South Fork Salmon River) to 15.4% (12.8–18.7%; upper Clearwater

River). The bulk of the run (65%) consisted of three reporting groups (Snake River–lower Clearwater River, upper Clearwater River, and lower Salmon River), with lesser contributions from the South Fork Salmon River, Middle Fork Salmon River, and upper Salmon River reporting groups. The reason for this disproportionate contribution is not immediately apparent. For example, the habitat of the Middle Fork Salmon River group is largely in protected wilderness with minimal anthropogenic impacts (Thurow 2000), yet the contribution of this group was relatively small (5.1%). Conversely, the Snake River–lower Clearwater River reporting group is from an area with relatively high human population densities and concomitant environmental disturbance. Contributions to the overall mixture were much lower for the Middle Fork Salmon River (mean = 5.1%; 95% CI = 3.5–6.4%) and South Fork Salmon River (2.7%; 1.3–3.6%) reporting groups. With an estimated run size of 25,764 wild steelhead migrating past Lower Granite Dam in SY 2009, approximately 1,314 (95% CI = 902–1,649) adults returned to the Middle Fork Salmon River and 696 (95% CI = 335–928) adults returned to the South Fork Salmon River. These estimates are below the critical population thresholds for these drainages as suggested by the ICBTRT (ICBTRT 2003) and are similar to escapement estimates proposed for these basins in the mid-1980s (Howell et al. 1985).

Beyond providing abundance estimates, our results suggest that the GSI methodologies applied to steelhead at Lower Granite Dam could contribute to documenting and monitoring a variety of diversity traits that are important for the viability of Snake River steelhead, including sex ratio, age and size at return, and run timing. We found that females comprised the majority (>65%) of the adult steelhead run passing Lower Granite Dam. This is not surprising because anadromy should benefit females more than males (Hendry et al. 2004). Sex ratios skewed toward females have been observed in adult steelhead populations throughout the species' range, including California, Alaska, the Columbia River basin, and the Kamchatka Peninsula in Russia (Savvaitova et al. 1997; Hendry et al. 2004; Christie et al. 2011; Hanson et al. 2011). Female-biased sex ratios in steelhead have been attributed to two separate life history behaviors: the predominance of residualization among males and the tendency of anadromous females to spawn more than once (Savvaitova et al. 1997; McMillan et al. 2007). Hydropower dams and distance from the ocean likely prevent most (if not all) successful iteroparous behavior in the Snake River basin (Keefer et al. 2008; Narum et al. 2008). The most likely explanation for the skewed sex ratios that we observed is the residualization of large numbers of males during freshwater rearing. This life history behavior may have been under a higher selective pressure over the last 40 years due to increased mortality associated with anadromy and may have helped to maintain the abundance and diversity of wild steelhead throughout the Snake River basin.

Our results, along with recent population genetic structure analyses (Nielsen et al. 2009), suggest that the reporting and management of Snake River steelhead by using designations based solely on fish length should be re-evaluated. The use of length criteria for stock delineation is clearly antiquated given the observed variation in freshwater and ocean residence periods and the evidence that all stocks produce both smaller-size or younger-age returning adults (i.e., A-run fish) and larger-size or older-age returning adults (i.e., B-run fish).

We believe that the GSI methodology that was employed to identify steelhead composition at Lower Granite Dam will prove to be an efficient and minimally intrusive tool for obtaining stock-specific abundance and life history information on Snake River steelhead. Small fin tissue samples can be obtained nonlethally from a subsample of returning adult steelhead each year, with minimal handling time and stress. In addition, almost all of the fish that we handled and sampled were successfully genotyped, thus indicating that few fish will undergo handling without ultimately contributing to GSI analyses. These are important considerations for monitoring efforts that involve an Endangered Species Act-listed species. Finally, the addition of an accurate genetic marker for sex provides new opportunities to examine sex-specific demographic processes that may influence population abundance and productivity.

Although the results of this initial study clearly demonstrate the possibilities of GSI technology as a tool for management and conservation of Snake River steelhead, there are still significant opportunities to improve the accuracy, precision, and efficiency of GSI techniques. Bias can be introduced into GSI estimation in several ways. If a significant portion of the escapement originates from populations that are not represented in the baseline, this will lead to misallocation and inaccurate contribution estimates. Because our baseline data set was constructed opportunistically from sampling and genotyping that were not specifically performed for GSI work in the Snake River basin, several important areas or drainages either were not represented or were underrepresented (i.e., North Fork Salmon River, Lolo Creek, Lemhi River, and upper Grande Ronde River). Future sampling should target these areas to determine their potential influence in genetic characterization of existing reporting groups or perhaps in redefining the reporting group delineations. Temporal sampling of the populations that are already included in our baseline will both increase sample sizes (improving allele frequency estimation) and test the stability of the baseline over time. In addition, as more samples become available, there will be increased opportunities for using known-origin individuals for independent testing of the baseline's accuracy beyond the simulation procedures performed here.

In addition to sampling-related issues, we are also interested in the utility of single-nucleotide polymorphic markers (SNPs) for improving GSI analyses in the Snake River basin. The SNPs are amenable for large-scale GSI efforts because they are abundant in the genomes of most organisms and are easily detected with recently developed DNA sequencing technologies (Metzker 2010). In addition, they are generally bi-allelic, which allows highly automated, rapid genotyping (Schlötterer 2004; Van Tassell et al. 2008; Seeb et al. 2009). Further, SNPs can be

found in the coding regions and *cis*—regulatory regions influenced by selection (Helyar et al. 2011), and research has shown that SNPs under diversifying selection may provide increased accuracy and precision in GSI analyses because these loci can exhibit higher differentiation among geographically proximate populations (Habicht et al. 2010; Ackerman et al. 2011).

We are currently working on expanding our sample and genetic marker baselines, and we expect that GSI methods will contribute substantially to future population viability assessments for steelhead in the Snake River basin, providing previously unavailable information on population abundance, productivity, spatial structure, and diversity.

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APPENDIX: Y-Chromosome-Specific Assays (*Omy_SEXY1* and *Omy_SEXY*)

The Y-chromosome-specific assays developed in this study to differentiate sex in steelhead were modified from markers developed by Brunelli et al. (2008) to run in two different configurations: as a TaqMan-based allelic discrimination assay and as a presence–absence assay in one of the multiplex microsatellite panels screened on steelhead. For the TaqMan-based allelic discrimination assay (*Omy_SEXY1*), we used published primers (Brunelli et al. 2008) and unpublished primers (J. Brunelli, Washington State University, personal communication) to sequence a Y-chromosome region

TABLE A.1. Quantity and concentration of PCR reagents used in the TaqMan-based allelic discrimination configuration of the Y-chromosome-specific assay for steelhead. Primer and probe sequences are also shown (6-FAM = 6-carboxyfluorescein; VIC = 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein; MGB = minor groove binder; NFQ = nonfluorescent quencher).

Quantity (μL)	Concentration	Reagent	Primer or probe sequence
5	10 ×	TaqMan Master Mix	
0.0225	100 μM	*OmyY1* probe e2	6-FAM-CCT ACC AAG TAC AGC CCC AA-MGB-NFQ
0.0050	100 μM	*OmyA* probe e500	VIC-GAG GGG TAG TCG TTT GTT CG-MGB-NFQ
0.0513	100 μM	OmyY1.4F primer	5′-CAC AAC ATG AGC TCA TGG G-3′
0.0513	100 μM	OmyY1.4R primer	5′-CGA TTA GAA AGG CCT GCT TG-3′
0.0100	100 μM	*OmyA* forward primer	5′-GCC TGC TTG CAG AAG TTT TT-3′
0.0100	100 μM	*OmyA* reverse primer	5′-CTT GAC TGT GTC CAG CTT GC-3′
3.8500	100 μM	Distilled H_2O	
1	Unknown	Template DNA	

(*OmyY1*; GenBank accession number EU081756) and to develop a 5′ exonuclease assay that amplifies a Y-specific product along with an autosomal product that acts as a control. These products are interrogated using fluorogenic probes (TaqMan chemistry, Applied Biosystems, Inc. [ABI], Foster City, California). Primer and probe sequences and PCR protocols for the TaqMan-based assay are summarized in Table A.1. Thermal cycling conditions were 95°C for 10 min followed by 55 cycles of 92°C for 15 s and 60°C for 1 min. Sex identification is accomplished through analysis of allelic discrimination plots of endpoint fluorescence using an ABI 7500 Real-Time PCR instrument (Figure A.1). The carboxyfluorescein (FAM) fluorophore (*y*-axis) is associated with the probe for the Y-specific product (males), while the 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) fluorophore (*x*-axis) labels the autosomal product. Samples that exhibit fluorescence from both FAM and VIC are scored as male. Samples that exhibit VIC fluorescence but not FAM fluorescence are scored as female. Samples that exhibit low or no fluorescence for both FAM and VIC are scored as "no call." The sex typing accuracy for *Omy_SEXY1* was evaluated by genotyping 135 known phenotypic male broodstock and

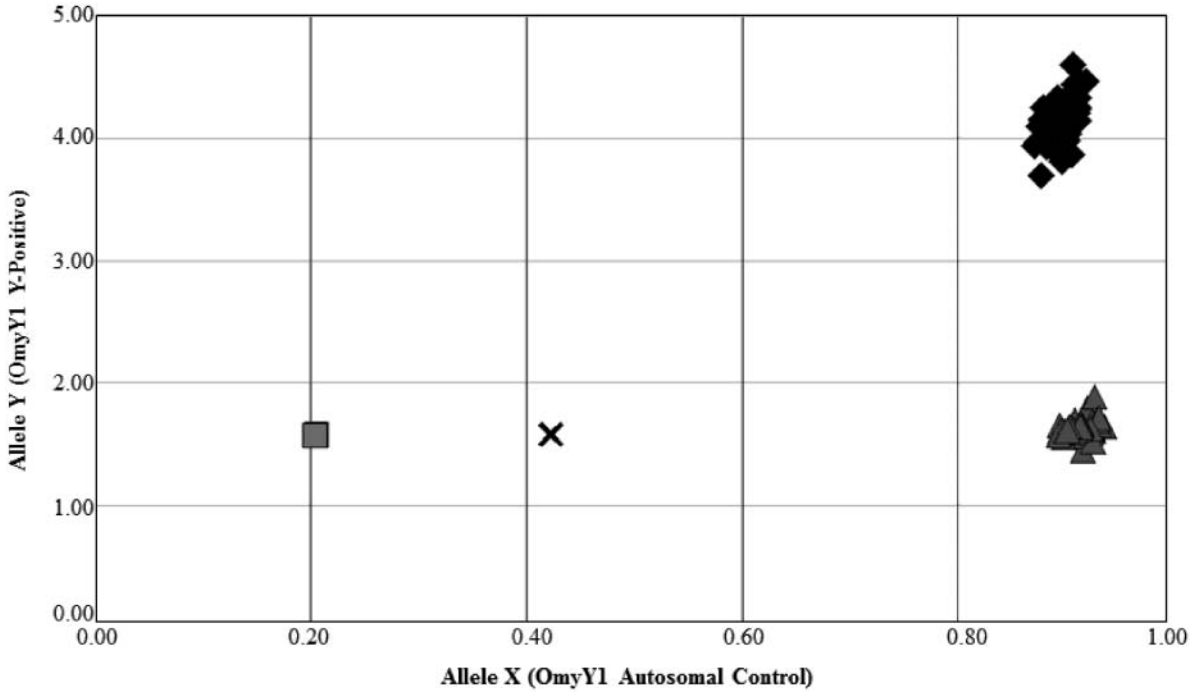

FIGURE A.1. An allelic discrimination plot, showing diagnostic clustering of male (diamonds) and female (triangles) steelhead by using a modified Y-specific assay (*Omy_SEXY1*). Samples identified by triangles amplified the autosomal *OmyA* locus only and are considered females. Samples identified by diamonds amplified both the autosomal *OmyA* locus and the Y-specific locus and are identified as males. The square represents a no-template control sample; the X represents a sample that failed to amplify properly and was not assigned a gender.

144 known phenotypic female broodstock from three Snake River steelhead hatcheries (Dworshak National Fish Hatchery, Sawtooth Fish Hatchery, and Wallowa Fish Hatchery). By following the procedures described above, 1 of the 135 known males was incorrectly identified as female, and the remaining 134 known males were correctly identified as males, thus yielding an overall accuracy of 99.2% (134/135). Of the 144 known females, 1 was scored as no call, 1 was incorrectly identified as male, and 142 were correctly identified as females. Based on the 143 samples scored (i.e., excluding the no-call sample), the overall accuracy for known females was 99.3% (142/143).

For the presence–absence assay (*Omy_SEXY*), we included a 5′-6-FAM fluorescently labeled, unpublished forward primer (OmyY1.2F; J. Brunelli, personal communication) and the reverse primer (OmyY1R) from Brunelli et al. (2008) with three microsatellite loci in a multiplex PCR amplification. Primer sequences, probe sequences, and PCR protocols for this assay are summarized in Table A.2. Thermal cycling conditions were 95°C for 15 min followed by 34 cycles of 94°C for 30 s, 57°C for 1 min 30 s, and 72°C for 60 s, and then a final extension of 60°C for 30 min.

The Y-chromosome-specific product amplified in this multiplex PCR was approximately 465 bp in length and was identified following capillary array electrophoresis using an ABI 3100 genetic fragment analyzer. The following rules were applied when conducting sex discrimination (Figure A.2): (1) any individual that amplified at the other loci in the panel and exhibited

TABLE A.2. Quantity of primer mix (concentration = 100 μM for all) used in the presence–absence multiplex PCR configuration of the Y-chromosome-specific assay for steelhead. Primer and probe sequences are also shown. Once the primer mix has been made, the PCR is run in a 5-μL volume on the 7500 Real-Time PCR instrument with 0.12 μL of primer mix, 2.50 μL of Qiagen Master Mix (catalog number 206143), 1.38 μL of distilled H_2O, and 1.00 μL of template DNA (unknown concentration; NED = 2′-chloro-5′-fluoro-7′,8′-fused phenyl-1,4-dichloro-6-carboxyfluorescein; 6-FAM = 6-carboxyfluorescein; VIC = 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein).

Quantity (μL)	Reagent	Primer or probe sequence
2.82	Omy1001F	5′-NED-GAT TCC ATA ACC TCG CCT TC-3′
2.82	Omy1001R	5′-GTC CTT GTG CTG CCT GCT-3′
2.12	Omy7F	5′-6-FAM-TTA AGT TTT GCC TAG ATA AGG G-3′
2.12	Omy7R	5′-CAA GGA ATG GCA CAG CTT G-3′
0.36	Ogo4F	5′-VIC-GTC GTC ACT GGC ATC AGC TA-3′
0.36	Ogo4R	5′-GAG TGG AGA TGC AGC CAA AG-3′
1.06	OmyY1.2F	5′-6-FAM-GCT AAT GGA CGA CGC TTT TC-3′
1.06	OmyY1R	5′-CGA TTA GAA AGG CCT GCT TG-3′

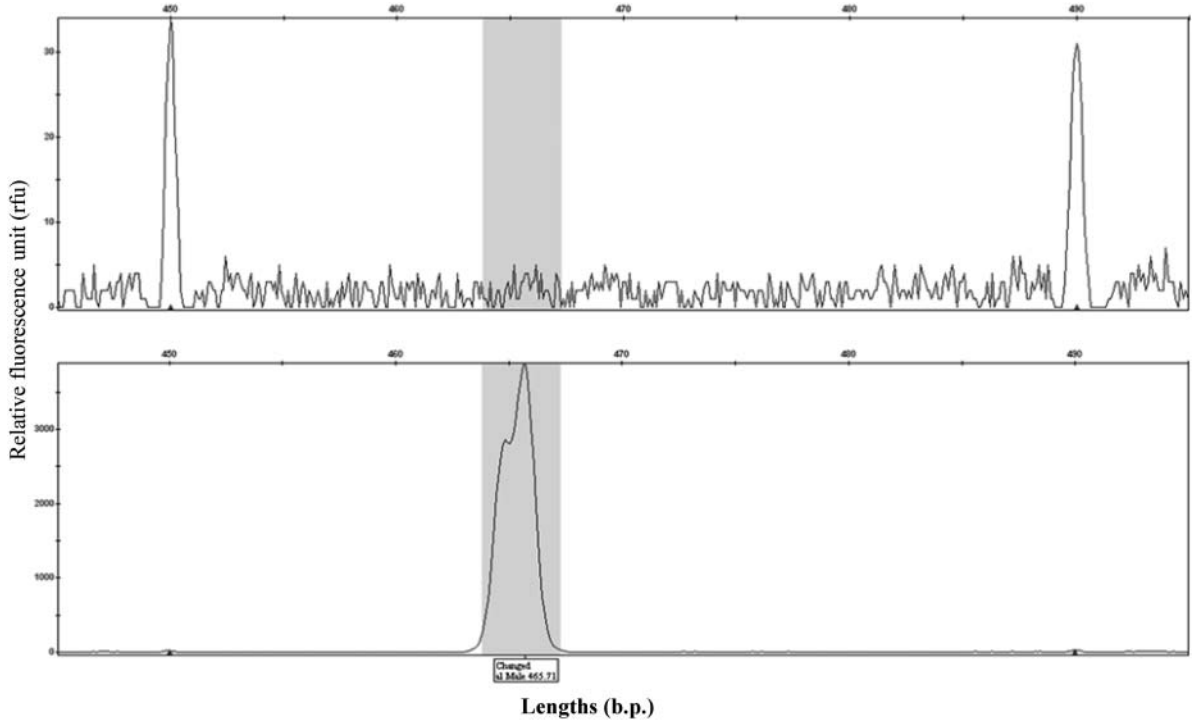

FIGURE A.2. Examples of electropherograms, showing absence (top) and presence (bottom) of the steelhead Y-chromosome-specific product (~465 bp) that was amplified in a multiplex PCR. The *y*-axis shows relative fluorescence units (RFUs), and the *x*-axis shows estimated length of the Y-chromosome-specific product (i.e., male "peak"). Samples with an observed peak greater than 1,000 RFUs were scored as male. Samples that exhibited a peak between 100 and 1,000 RFUs were scored as "no call."

an *OmyY1* peak greater than 1,000 relative fluorescence units (RFUs) was scored as male; (2) samples that exhibited a peak between 100 and 1,000 RFUs were scored as no call; (3) samples that failed to amplify at the other loci in the panel were scored as no call regardless of the peak-height RFUs at *OmyY1*; and (4) any individual that amplified at the other loci in the panel and exhibited either no peak or a peak less than 100 RFUs was scored as female. The sex typing accuracy for *Omy_SEXY* was evaluated by genotyping 630 known phenotypic male broodstock and 297 known phenotypic female broodstock from the Oxbow Fish Hatchery. Using the scoring rules described above, 4 of the 630 known males were scored as no call, 5 were incorrectly identified as females, and 621 were correctly identified as males. Based on the 626 samples scored (excluding the no-call samples), the overall accuracy for known males was 99.2% (621/626). Of the 297 known females, 7 were scored as no call, 4 were incorrectly identified as males, and 286 were correctly identified as females. Based on the 290 samples scored, the overall accuracy for known females was 98.6% (286/290).

In this study, we screened all adult samples from Lower Granite Dam by using the presence–absence configuration of the Y-chromosome-specific assay. We also screened a total of 327 samples by using the TaqMan-based allelic discrimination configuration of the assay. For the 327 samples in which both assay configurations were run, concordance was high (99.4%; 325/327).